Theor Appl Genet (1988) 76:669–672

Dominance relationships among S-alleles in *Corylus avellana* L.*

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Received June 21, 1988; Accepted June 25, 1988
Communicated by H. F. Linskens

Summary. Pollen-stigma compatibility relationship were studied in 50 cultivars and more than 800 seedlings of the European hazelnut (*Corylus avellana* L.). A total of 22 unique S-alleles have been identified. Dominance relationships in 75 of the possible 231 pairs of alleles have been determined in both pistil and pollen. In the pistil, all alleles exhibited independent action, whereas in the pollen, alleles exhibited either dominance or codominance. The dominance relationship was linear with 7 levels of dominance.

Key words. Incompatibility – Hazelnut – Filbert – *Corylus* – S-alleles

Introduction

Self-incompartibility is a widespread phenomenon which promotes outcrossing in flowering plants. In a hazelnut (*Corylus avellana* L.) breeding program, incompatability prevents making many desirable crosses in some cases, while in other cases it dictates the direction of the cross. Recommendations for new plantings require the identification of mutually compatible cultivars (cvs). In hazelnut, incompatibility is determined sporophytically by a single locus with multiple alleles (Thompson 1979 a). The stigmatic surface is the site of the incompatibility reaction.

A one-locus sporophytic incompatibility system with multiple alleles is common in the Compositae and the Cruciferae. In some species, S-alleles express dominance in both pollen and pistil, and the dominance relationship is generally linear, as in *Ageratum houstonianum* (Stephens et al. 1982), *Cosmos bipinnatus* (Crowe 1954), *Iberis amara* (Bateman 1954), and *Cichorium intybus* (Eenink 1981). In others, dominance among S-alleles is expressed only in the pollen and is generally linear, as in *Partenium argentatum* (Gerstel 1950), *Crepis foetida* (Hughes and Babcock 1950), and *Carthamus flavescens* (Imrie and Knowles 1971). However, the situation in *Brassica* and *Raphanus* is more complex. Dominance may be expressed in both pollen and pistil. Pairs of alleles may exhibit complete dominance, partial dominance, codominance, or mutual weakening. Dominance relationships may be linear or nonlinear and may be different in pollen and pistil. In general, partial dominance is more common than complete dominance, and dominance is expressed more clearly in the pollen than in the stigma (Sampson 1967; Richards and Thurling 1973; Wallace 1979).

In a previous report (Thompson 1979 b), 11 alleles were identified in hazelnut. The objective of the present study was to locate additional S-alleles and to determine the dominance relationships among them.

Material and methods

The cultivars and seedling used for these studies are part of a breeding program at Oregon State University (OSU), Corvallis OR. Originally from various countries in Europe and Turkey, they constitute a very diverse gene pool. When staminate catkins had elongated and were about to shed, pollen was collected and stored in cotton-stoppered vials in the freezer (–20 °C). Female flower clusters, which consists of several stigmatic styles emerging from the apex of a dormant bud, were generally collected from bagged branches. However, flowers from unbagged branches were also used if the were bright red, indicating that they had only recently emerged, and that open pollination had not occurred. Pollination induces darkening in style color in less than 12 h. Clusters were detached from limbs when styles protruded 2–6 mm and placed in Petri dishes on moist filter paper. Tester parents, whose alleles had previously been established, are listed in Table 1. They were used as both male and female parents in these controlled pollinations. Pollinations were performed in the laboratory as soon as possible after collection by dipping the flower clusters in the appropriate vial of pollen and leaving them in the covered Petri dish overnight. For each cross,

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* Oregon Agricultural Experiment Station Technical Paper No. 8542

Table 1. Testers for S-alleles. Alleles expressed in the pollen are underlined

Allele	Tester	S-alleles
1	Barcelona	$\underline{1}$ 2
	Montebello	$\underline{1}$ 2
2	OSU 20-58	$\underline{2}$ $\underline{2}$
3	Nonpareil	1 $\underline{3}$
4	OSU 194-1	$\underline{4}$ $\underline{4}$
5	Badem	2 $\underline{5}$
6	Henneman # 3	$\underline{6}$ 10
7	Tonda Gentile delle Langhe	2 $\underline{7}$
8	Tombul Ghiaghli	4 $\underline{8}$
9	Segorbe	$\underline{9}$?
10	Imperiale de Trebizonde	2 $\underline{10}$
11	OSU 28-91	4 $\underline{11}$
12	Tombul (=Extra Ghiaghli)	4 $\underline{12}$
13	USOR 98-83	6 $\underline{13}$
14	Gem	2 $\underline{14}$
15	Italian Red	$\underline{15}$ $\underline{20}$
	Hall's Giant	$\underline{5}$ $\underline{15}$
16	Ribet	2 $\underline{16}$
17	Mortarella	2 $\underline{17}$
18	Neue Riesennuss	$\underline{18}$?
	Romisondo G-1	$\underline{18}$ $\underline{20}$
19	OSU 92-43	11 $\underline{19}$
20	Italian Red	$\underline{15}$ $\underline{20}$
	Romisondo G-1	$\underline{18}$ $\underline{20}$
	Tonda Romana	$\underline{10}$ $\underline{20}$
21	OSU 168-26	2 $\underline{21}$
22	OSU 219-133	4 $\underline{22}$

2–3 flower clusters, each consisting of several styles, were pollinated. Most flowers were squashed the following morning in aniline blue dye (0.1 g aniline blue, 0.71 g K_3PO_4, 100 ml distilled water) and examined with a fluorescence microscope. Others were placed in the freezer until convenient to analyze. In an incompatible cross, pollen germination is variable and dependent on female flower quality. Pollen grains that germinate produce short tubes which fail to penetrate the stigmatic surface. These tubes fluoresce strongly and typically terminate in a pronounced bulb. Compatible pollen germinates well, and tubes penetrate the styler tissue, producing a mass of long parallel tubes with strongly fluorescing callose plugs. When fresh, unpollinated female flowers and viable tester pollen are used, the two reactions are quickly and easily distinguished.

In 1979, only 11 S-alleles had been identified. The search for new S-alleles and new conbinations of S-alleles started with the collection of pistillate clusters from previously identified testers and pollen from cultivars of unknown parentage, and making several series of crosses. An incompatible reaction indicated that a common allele was expressed in the pollen and the female flower. However, if the cross was compatible on all female testers, it was concluded that 1 or 2 new S-alleles were expressed in the pollen and, thus, that a new pollen tester had been established. Pistillate clusters from the potential new tester trees were then pollinated with all pollen testers. A compatible reaction with all pollen testers indicated the presence of 1 or 2 new S-alleles. A single incompatible reaction indicated the presence of one known and one unknown allele. Reciprocal crosses were then made to confirm that the new allele was dominant in the pollen. Other S-alleles were recessive in named cultivars (S_{19} and S_{22}). Pollen testers were identified in their seedlings by the procedure outlines above. New alleles were identified and new testers added sequentially.

Dominance relationships between new combinations of S-alleles in seedlings from controlled crosses between parents with known S-alleles were established by first collecting female flower clusters from the seedlings and then testing them for the presence of parental alleles (usually 4). Once the two alleles in each

Table 2. Dominance relationships between pairs of S-alleles

Allele in question	Alleles dominant to the allele in question	Alleles codominant with the allele in question	Alleles recessive to the allele in question
1	3, 6, 8	5, 7, 10, 12, 14, 15, 18, 20, 21	2, 4, 11, 19
2	1, 3, 5, 6, 7, 8, 10, 12, 14, 16, 17, 21		4, 11, 22
3		8, 9	1, 2, 4, 5, 7, 10, 11, 12, 14, 15, 20, 21
4	1, 2, 3, 5, 7, 8, 10, 11, 12, 20, 22		
5	3	1, 10, 15	2, 4, 11
6	13		1, 2, 10, 11, 14, 19
7	3, 8	1, 10, 12	2, 4, 11
8		3	1, 2, 4, 7, 10, 12
9		3	
10	3, 6, 8	1, 5, 7, 18, 20, 21	2, 4, 11, 22
11	1, 2, 3, 5, 6, 7, 10, 15, 19		4
12	3, 8	1, 7, 21	2, 4
13			6, 19
14	3, 6	1	2
15	3	1, 5, 20	11
16			2
17			2
18		1, 10, 20	
19	1, 6, 13		11
20	3	1, 10, 15, 18	4
21	3	1, 10, 12	2
22	2, 10		4

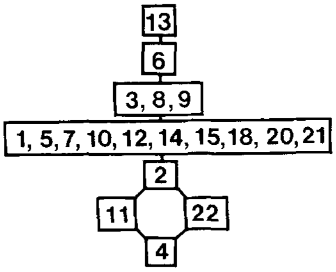

Fig. 1. Dominance hierarchy in hazelnut pollen. Alleles are dominant to alleles below them, and codominant with those in the same box

seedling were identified, pollen was collected from each new combination. This pollen was placed on female flowers of the two appropriate testers, each bearing only one of the two pertinent alleles and a different allele. Pollen incompatibility on only one tester indicates dominance of the common allele, whereas incompatibility on both female testers indicates codominance of the two alleles.

Results

An additional 11 S-alleles have been identified since the last report (Thompson 1979 b), bringing the total number of available pollen testers to 22. The recessive allele of 'Tombul' was incorrectly designated S_{13} in an earlier paper (Thompson et al. 1985). It is S_4 as is the recessive allele of 'Tombul Ghiaghli'.

Dominance relationships between 75 of the possible 231 pairs of alleles were identified (Table 2). Although both alleles were always expressed in the pistils, allelic pairs exhibited dominance or codominance in the pollen. 'Tombul', 'Gem', 'Ribet', 'Mortarella', and 'Neue Riesennuss' were found to have new dominant alleles and were added to the list of testers. Instances in which pollen testers proved to have codominant alleles were also discovered. For example, 'Hall's Giant' was reciprocally cross-incompatible with both 'Badem' and 'Italian Red', yet 'Badem' and 'Italian Red' were reciprocally cross-compatible, indicating that 'Hall's Giant' shared a different codominant allele with each of the other two cultivars. Likewise, 'Tonda Romana' was reciprocally cross-incompatible with 'Imperiale de Trebizonde' and 'Italian Red', yet the latter two clones were reciprocally cross-compatible. Since 'Badem' was known to be S_2 S_5 and 'Imperiale de Trebizonde' to be S_2 S_{10}, 'Hall's Giant' was assumed to be S_5 S_{15}, 'Tonda Romana' to be S_{10} S_{20}' and 'Italian Red' to be S_{15} S_{20}.

Based on the specific dominance relations listed in Table 2, 19 alleles can be placed in a linear dominance hierarchy with 7 levels of dominance (Fig. 1). The other three alleles have not yet been combined with enough other alleles to place them in the scheme. S_{11} and S_{22} are both recessive to S_2, but dominant to S_4, yet they, themselves, have not been paired in the same tree so their relationship to each other is unknown. This pairing, along with the additional information required to place S_{16}, S_{17}, and S_{19} in the scheme, may lead to the discovery of additional dominance levels.

Table 3. S-alleles of hazelnut cultivars. Alleles expressed in the pollen are underlined

Cultivar	S-alleles
C. avellana var fusco rubra	$\underline{6}$ 19
C. avellana var contorta	$\underline{5}$ $\underline{10}$
Badem	2 $\underline{5}$
Barcelona	$\underline{1}$ 2
Brixnut	$\underline{1}$ $\underline{14}$
Butler	2 $\underline{3}$
Campanica	$\underline{1}$ 2
Casina (W4-6)	$\underline{10}$ $\underline{21}$
Compton	2 $\underline{3}$
Cosford	$\underline{3}$?
Creswell	2 $\underline{10}$
Daviana	$\underline{3}$ 11
DuChilly (Kentish Cob)	$\underline{8}$ 10
Ennis	$\underline{1}$ 11
Fitzgerald # 20	$\underline{2}$ 11
Gasaway	$\underline{3}$?
Gem	2 $\underline{14}$
Hall's Giant (Bolwyller)	$\underline{5}$ $\underline{15}$
Henneman # 3	$\underline{6}$ 10
Imperatrice Eugenie	$\underline{3}$ 14
Imperiale de Trebizonde	2 $\underline{10}$
Italian Red	$\underline{15}$ $\underline{20}$
Kruse	$\underline{1}$ 2
Lansing # 1	1 $\underline{3}$
Lansing # 2	$\underline{3}$ 10
Montebello	$\underline{1}$ 2
Morell	$\underline{1}$ 2
Mortarella	2 $\underline{17}$
Negret	$\underline{10}$ 22
Neue Riesennuss	$\underline{18}$?
Nonpareil	1 $\underline{3}$
Nooksack	$\underline{6}$ 14
Nottingham	$\underline{8}$ 10
Pallaz	$\underline{2}$?
Red Lambert	$\underline{5}$ $\underline{10}$
Ribet	2 $\underline{16}$
Riccia di Talanico	$\underline{1}$ 2
Rode Zeller	$\underline{6}$?
Royal	1 $\underline{3}$
Segorbe	$\underline{9}$?
Sivri Ghiaghli	$\underline{12}$?
Tombul (Extra Ghiaghli)	4 $\underline{12}$
Tonda di Biglini	$\underline{1}$ $\underline{10}$
Tonda di Giffoni	$\underline{2}$?
Tonda Gentile delle Langhe	2 $\underline{7}$
Tonda Romana	$\underline{10}$ $\underline{20}$
White Aveline	$\underline{5}$ $\underline{10}$
Woodford	1 $\underline{3}$

Discussion

The availability of a tester for each of the 22 S-alleles has permitted a more through study of the incompatibility alleles present in common cultivars (Table 3). Additional alleles will undoubtedly be identified as the collection grows. For example, the second alleles of 'Cosford', 'Gasaway', 'Neue Riesennuss', 'Rode Zeller', 'Pallaz', 'Segorbe', 'Sivri Ghiaghli', and 'Tonda di Giffoni' remain unidentified, although they have been tested for most of the known S-alleles. 'Gasaway' and 'Segorbe' transmit their unidentified second alleles to half of their progeny.

Knowledge of the dominance hierarchy in hazelnut pollen allows for the rapid isolation of new testers. As new cultivars are added to the OSU collection, they are tested against all known alleles. When this fails to identify two alleles, crosses are made with selections carrying S_4. Since S_4 is recessive to all known S-alleles, new alleles can be identified, and new testers become available in the first generation. This approach led to the identification of S_{22} and OSU 219-133 as a tester for it. Homozygotes are possible in the sporophytic system, and have been obtained for alleles S_1, S_2, and S_4. Cultivars with only one identified S-allele may be homozygous. Progeny testing is necessary to determine whether they are homozygous, or whether the other allele is a new allele for which no pollen tester yet exists, or whether there is dominance in the female. The latter situation is unlikely, as more than 800 seedlings from controlled crosses have been checked, and in each case an allele from each parent has been expressed in the pistil.

Knowledge of the dominance relationships among S-alleles is also helful in the creation of homozygous testers. For example, a homozygote for S_6 can only be obtained by crossing a female carrying S_6 with a S_6 S_{13} pollen parent. Similarly, S_3, S_6, S_8, or S_9 could be used to obtain a homozygote for S_{10}. Homozygous testers would simplify allele identification and be useful in basic pollination and incompatibility studies.

The dominance relationship among S-alleles in hazelnut resembles that of *Parthenium argentatum, Crepsis foetida,* and *Carthamus flavescens,* all members of the Compositae. Many of the early studies used populations with a very narrow genetic base and a few S-alleles. The OSU hazelnut collection has a broad genetic base. As additional combinations of alleles are obtained, dominance in the stigma or nonlinear dominance in the pollen may be identified, and the model accordingly adjusted. At this time, however, the sporophytic incompatibility system in hazelnut appears to be among the simplest of those studied.

Acknowledgements. This research was supported by the Oregon Hazelnut Commission.

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